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Understanding and Controlling Living/ Inorganic Interfaces to Enable Reconfigurable Switchable Materials

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14. ABSTRACT Results are highlighted for a 3-year study to study fundamental interactions between engineered <i>E. coli</i> cells and inorganic surfaces (gold and functionalized silica) to develop tailored, switchable bacterial adhesion to a target surface. This work includes protein engineering of multiple targets (FimH and eCPX), a variety of analytical techniques (including scanning electron microscopy and atomic force microscopy), theoretical models including multiphysics and multiscale treatments, as well as one of the first known studies of the role of environmental conditions and surface treatment on binding affinity.					
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1. Introduction and Research Objective

The integration, organization, and control of biological components into hybrid materials will allow the development of novel system properties and advanced functionality in plasmonics, optics, catalysis, biosensing, power generation, and energy storage, among a myriad of other applications. Our research moves toward these goals by developing control of bacterial adhesion on inorganic surfaces sufficient to revolutionize hybrid device development with the invention of tailored switchable binding on a target surface (the primary objective of the project). Future work can build upon this to focus on autonomous and directed patterning and reconfigurable binding, self-healing properties, responsive properties and more advanced forms of switching to control mass transfer and electron transfer at the interface.

2. Research Strategy

We leverage existing US Army Research Laboratory (ARL) strengths (e.g., molecular biology/synthetic biology, biomolecular recognition, materials characterization and polymer science, computational biology) and facilities (DOD Supercomputing Resource Center, ARL Specialty Electronics Materials and Sensors Cleanroom, biomaterial and polymer material characterization capabilities, and biotechnology laboratories). The pathway followed was the integrated development of switchable, controllable bacterial adhesion by investigation of 2 families of switches (chemical/environmental and DNA programmed) acting on 2 modes of binding in *E. coli* (fimbrial protein [FimH] and coat protein [eCPX]) on 2 target surfaces (gold [Au] and functionalized silicon [Si]).

3. Research Highlights

The research focus through the 3-year course of this work has covered multiple binding modes, multiple surfaces, and a variety of factors related to putative binding switches, as outlined in the research strategy. Highlights include:

- 1) First-ever demonstration of selective cell autotemplating, demonstrating directed assembly through functional and selective activity of 3 phage-derived Au-binding sequences in the *E. coli* bacterial cell surface display scaffold (eCPX). Preferential binding of engineered *E. coli* to Au over Si by up to 4 orders of magnitude was obtained. Further, functional activity post assembly was preserved, a key criterion for future living composite materials and systems.

- 2) Development of a 30+ chemical on-switch for *E. coli* adhesion to Au through arabinose-induced expression of bacterial eCPX.
- 3) Successful demonstration of engineered fimbrial (FimH) adhesion of *E. coli* to Au surfaces. Successful iteration between experiment and theory leads to improved location of insertion site for Au-binding sequence in FimH protein, demonstrating almost complete coverage of the target surface and up to 10- μ m resolution of surface patterning. This is achieved solely through bacterial capture, and does not require any growth or amplification processes.
- 4) Successful demonstration of *E. coli* adhesion to functionalized (mannosylated) Si and tested under a variety of flow (shear) conditions to test for the putative “catch bond”.
- 5) First known study to address environmental conditions and surface treatment on binding affinity, carried out by X-ray photoelectron spectroscopy (XPS) and spot assay.
- 6) Successful theoretical analysis of energetics of binding and validation of classical methods.
- 7) Successful elucidation of contributions of eCPX components (N terminus/binding sequence/C terminus) to binding affinity.
- 8) Successful comparison of whole cell-binding mode (FimH vs. eCPX) by spot assay and atomic force microscopy (AFM).
- 9) Successful study of “living” dynamics of binding of filamentous bacteria under shear and development of COMSOL model.
- 10) Successful integration of both engineered scaffold systems for Au into microbial fuel systems showing enhanced output.
- 11) Successful development of chemical off-switch—chemically induced unbinding in peptide-mediated (eCPX) bound *E. coli* on Au surface. Proof of concept achieved.

4. Applications Underway

We have studied the behavior of filamentous *E. coli* on surfaces under shear and demonstrated controlled growth of filamenting cells to form a living bridge between Au domains on patterned glass. Bacterial systems developed under this program have been used to develop successful microbial fuel cells (MFCs), and have further

delineated differences between binding modes and furthered the understanding of overall cell performance that is correlated to scaffold engineering. Additional work has been performed studying the interactions between these bacterial systems and gold nanoparticles (AuNPs). While the metal coverage of the bacterial cells achieved stopped short of levels needed for production of programmed, autonomous circuit assembly and living, rehealing wires/components, there are nonetheless implications for directed drug delivery and catalysis, and more generally for dynamic responsive material coatings and composites.

5. Army Impact

The ability to create artificial biological systems and controllably interface these materials with nonliving inorganics is expected to broadly impact products in diverse areas such as bioenergy, biosensors, bioadhesives, vaccines, programmable devices, reconfigurable and self-healing materials, transient electronics, living-anti-corrosion paints, high-strength materials inspired by nature, robust human-machine interfaces, smart skins, ubiquitous sensing, and Soldier augmentation.

6. Challenges and Lessons Learned

Not surprisingly, most challenges are created by the inherent complexity/fragility/evolutionary capacity of the biological system, and the widely variable properties of the target surface due to morphological intricacies that influence underlying quantum effects. Subtle changes in the surface properties can have large repercussions with interactions both on- (eCPX or Fimh) and off-scaffold. This is simultaneously both a challenge (to minimize interference or loss of function) and a potential tool to employ as we move from static to more dynamic living material systems. To this end, it is clear that a hybrid biological and materials science approach is needed with a systems-level view of the material composite.

It is difficult to adapt existing characterization tools to dynamic, complex, and living material systems, which require a full complement of methods bridging scales both experimentally and by simulation. Pushing the limits on the fundamental characterization methods as well as simulation algorithms requires extremely large data sets as well as a plethora of controls. To mitigate this risk, we take a holistic systems approach and work fundamentally across scales, and in a manner to bridge these scales working iteratively between components and the complex materials in a systematic way.

It is difficult to perform in situ chemical synthesis without adversely affecting viability and function of the bacteria. In particular metal salts are a challenge and it may require using biomineralizing bacteria up front for tailored applications.

Biological systems, especially bacteria, evolve over time through random genetic mutations, which alter fundamental behavior. This can have positive (additive effects) or deleterious consequences on the material/system performance. It is clear that this is a largely unexplored area, especially toward military applications/environments.

7. Conclusion and Beyond

This work represents significant progress toward the goal of tailored switchable binding of bacterial (*E. coli*) cells to an inorganic surface. Multiple chemical on-switches have been demonstrated for both fimbrial (FimH) (see Figs. 1 and 2) and membrane surface protein (eCPX) (see Fig. 4) adhesion. A multiphysics COMSOL model was developed in the course of studying the living dynamics of filamentous bacteria under shear (see Fig. 3). Chemically induced unbinding of an engineered *E. coli* cell bound to an Au surface was achieved as a successful proof of concept. As a demonstration of applicability, the resulting engineered bacterial scaffolds have been integrated into microbial fuel systems and show enhanced output. As part of the Director's Strategic Initiative, this work is highly relevant to ongoing studies within the Biotechnology Branch. In FY18 this program has transitioned to an existing 6.1 funding line for Engineered Biology. In FY19 and beyond, this work will transition into the new Living Materials program to enable reconfigurable, dynamic living materials.

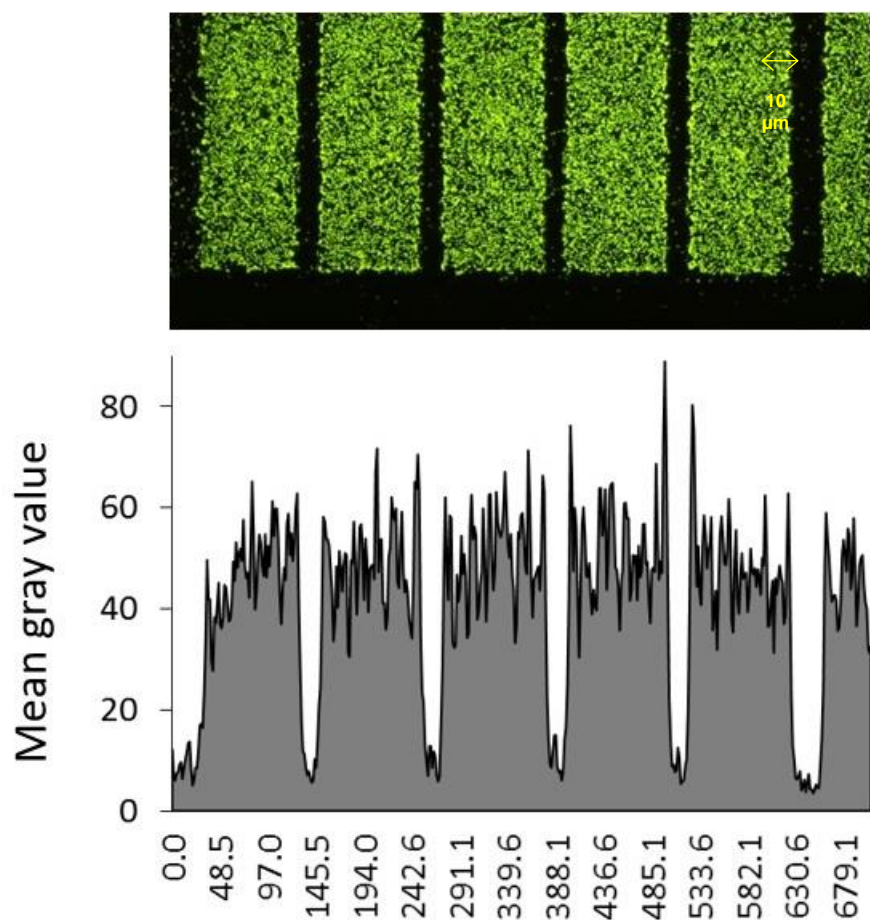


Fig. 1 Demonstration of Au-binding properties by cells expressing mFimH. The above image shows *E. coli* cells engineered FimH (M6G4 added as a binding sequence) adhering with 10-μm resolution to Au patterned onto silica.

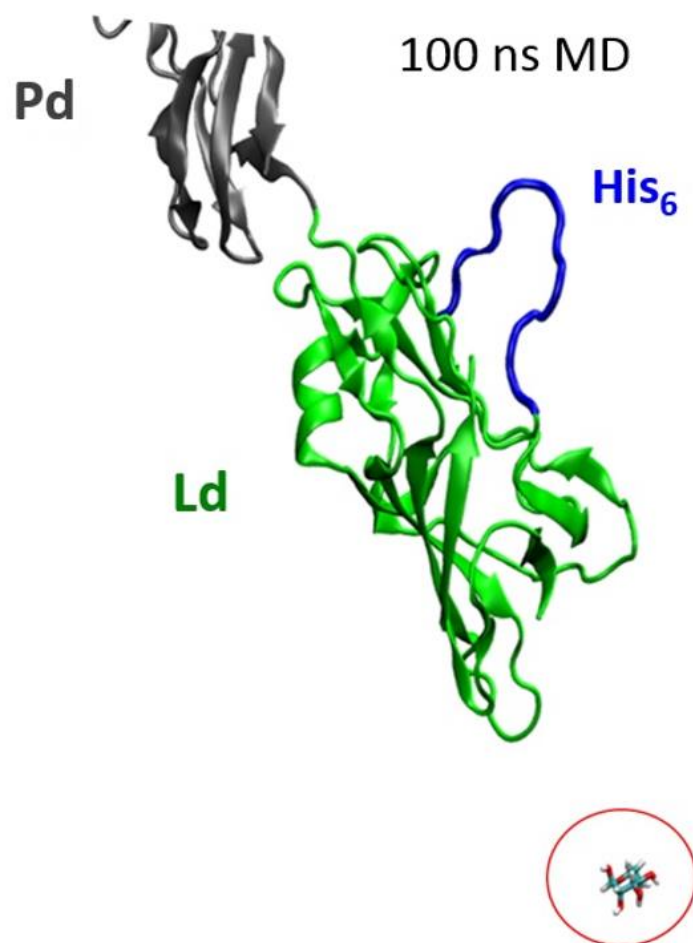


Fig. 2 Engineered vFimH exposes a His6 tag at ILE52 in the lectin-domain (Ld) and seen to disrupt mannose binding both experimentally and in molecular dynamics simulation

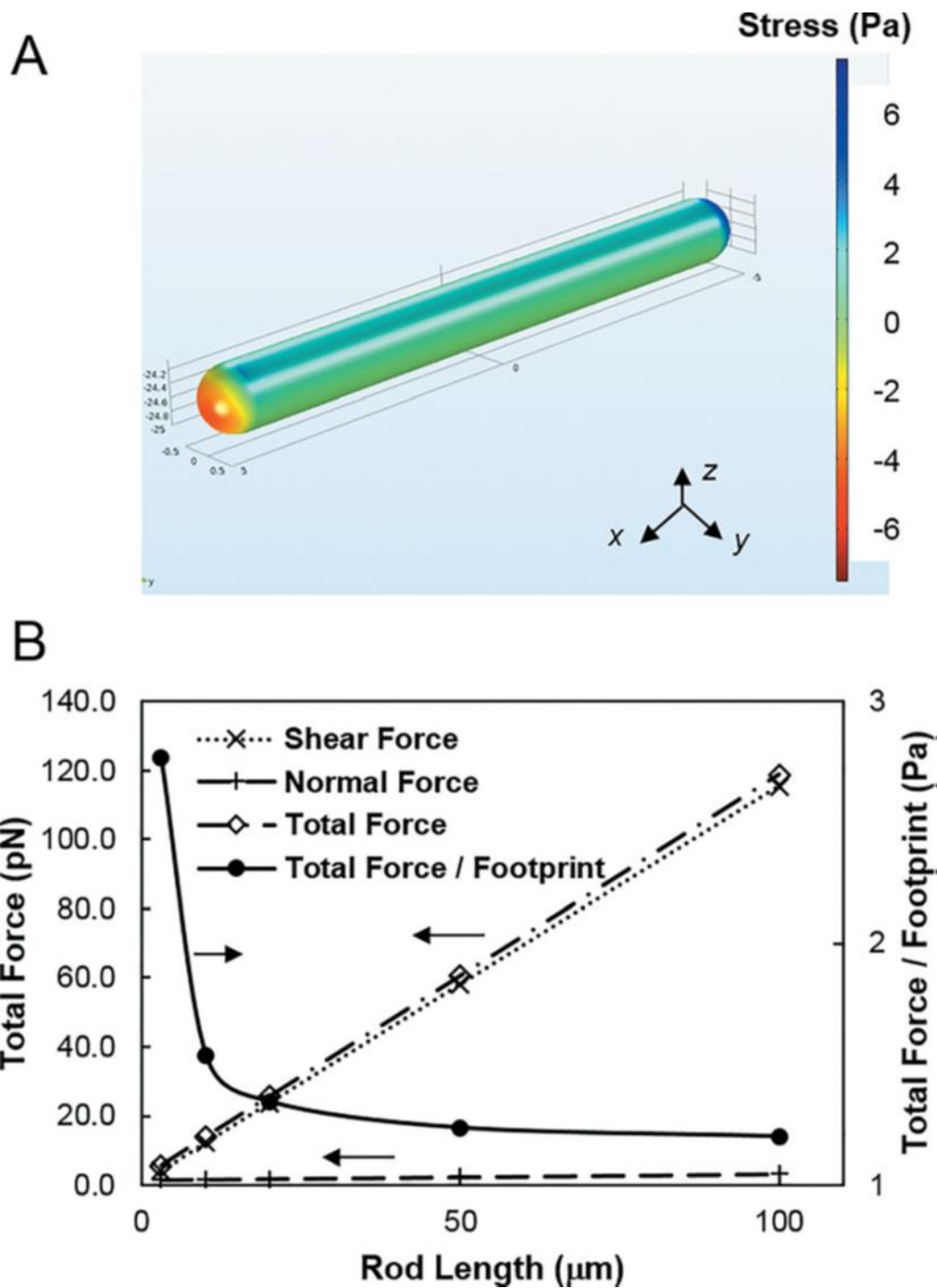


Fig. 3 COMSOL Multiphysics model demonstrating normal and total forces exerted by fluid flow on bacterial cell (modeled as a rigid rod). Image is taken from Jahnke et al. *Biointerphases* 12, 02C410 (2017).

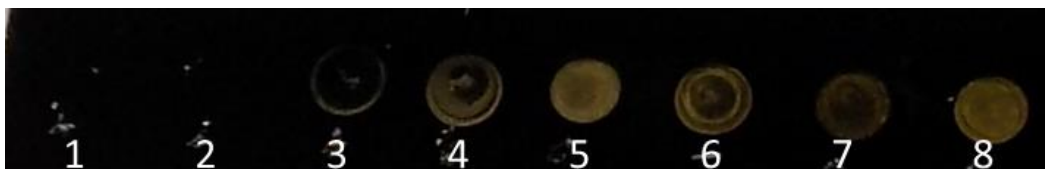


Fig. 4 Example of gold binding spot assay for *E. coli* cells (uninduced cells in lane 1) induced to express the eCPX peptide display scaffold (peptide-free scaffold in lane 2) with various N-terminal peptides for binding to gold (lanes 3–8: examples of designed peptides and peptides from literature in lanes).

Appendix. Publications and Presentations

Publications and Presentations

- Adams BL, Hurley MM, Jahnke JP, Stratis-Cullum DN. Functional and selective bacterial interfaces using cross-scaffold gold binding peptides. *JOM*. 2015 Nov;67(11):2483–2493. DOI: 10.1007/s11837-015-1662-7. Invited.
- Adams BL, Hurley MM, Sarkes DA, Stratis-Cullum D. Bacterial surface display for discovery and study of material specific peptides. *Proteins and Cells at Interfaces*, 2015 Annual Society for Biomaterials Meeting; 2015 Apr 15–18; Charlotte, NC.
- Adams BL, Stratis-Cullum DN. Selective preference of engineered peptides for an aluminum alloy. *Emerging Mater Res*. 2015;4:297–310. Invited.
- Dong H, Sarkes DA, Rice JJ, Hurley MM, Fu A, Stratis-Cullum D. Functionalization of living bacterial cells with metallic nanoparticles mediated by surface-displayed peptides. *American Chemical Society 254th National Meeting*; 2017 Aug; Washington, DC. Oral presentation.
- Dong H, Sarkes DA, Rice JJ, Hurley MM, Fu A, Stratis-Cullum D. Living composites: Viable bacteria-nanoparticle hybrids mediated through surface-displayed peptides. *Langmuir*. Forthcoming 2017.
- Dong H, Sarkes DA, Rice JJ, Hurley MM, Fu A, Stratis-Cullum DN. Living bacteria/nanoparticle hybrids mediated by surface-displayed peptides. *ARAP workshop and review*. 2017 Sep.
- Hurley MM. Computational studies of peptide-surface interactions drawn from bacterial display studies: up close and personal. Presented at the *Bio-Nano Interfaces and Engineering Applications Symposium*, 2017 TMS National Meeting; 2017 Feb; San Diego, CA. Invited talk.
- Jahnke JP, Smith AM, Zander NE, Wiedorn V, Strawhecker KE, Terrell JL, Stratis-Cullum DN, Cheng X. “Living” dynamics of filamentous bacteria on an adherent surface under hydrodynamic exposure. *Biointerphases*. 2017;12(2):02C410. DOI: <http://dx.doi.org/10.1116/1.4983150>. Invited.
- Jahnke JP, Terrell JL, Smith AM, Chen X, Stratis-Cullum DN. Influences of adhesion variability on the ‘living’ dynamics of filamentous bacteria in microfluidic channels. *Molecules*. 2016 July;21(8):E985. DOI: 10.3390/molecules21090985.
- Jahnke JP, Terrell JL, Smith AM, Cheng X, Stratis-Cullum DN. Spatiotemporal dynamics of filamentous bacteria near and on affinity substrates. *American*

Chemical Society 254th National Meeting; 2017 Aug; Washington DC. Oral presentation.

Small MC, Stratis-Cullum DN, Adams BL, Jahnke JP, Sarkes DA, Dong H, Terrell JL, Hurley MM. Understanding the effect of amino acid conformation on binding affinity to Au(111) using quantum mechanical calculations. 252nd American Chemical Society National Meeting; 2016 Aug 21; Philadelphia, PA.

Small MC, Stratis-Cullum DN, Adams BL, Jahnke JP, Sarkes DA, Dong H, Terrell JL, Hurley MM. Understanding the effect of amino acid conformation on binding affinity to Au(111) using quantum mechanical calculations. Poster presentation at the American Chemical Society 254th National Meeting; 2017 Aug; Washington, DC.

Stratis-Cullum DN. From materials science to next generation sensing using synthetic and engineered peptide technology. SPIE DSS; 2015 Apr 24; Baltimore, MD. Paper 9487-24. Invited.

Stratis-Cullum DN, Adams BL. Discovery of peptides developed using bacterial surface display. MS&T; 2014 Oct; Pittsburgh, PA. Invited.

Technical Assessment Board (TAB) poster presentations FY15.

Technical Assessment Board (TAB) oral presentation FY15.

Terrell JL, Adams B, Dong H, Jahnke J, Sarkes D, Small M, Gerlach ES, Liu S, Hurley M, Stratis-Cullum D. Living Hybrid Materials. ARAP workshop and review; 2017 Sep.

Terrell JL, Dong H, Holtoff EL, Small MC, Sarkes DA, Hurley MM, Stratis-Cullum DN. Investigation of engineered bacterial adhesins for opportunity to interface cells with abiotic materials. Smart Biomedical and Physiological Sensor Technology XIII Proc. of SPIE; 2016. vol. 9862, 986308.

Terrell JL, Dong H, Holtoff EL, Small MC, Sarkes DA, Hurley MM, Stratis-Cullum DN. Switchable interfacing between living and abiotic materials engineered bacterial adhesion. SPIE 2016: Defense and Commercial Sensing; 2016 Apr 18; Baltimore, MD.

Terrell JL, Dong H, Small MC, Sarkes DA, Jahnke JP, Hurley MM, Bentley WE, Stratis-Cullum DN. Alteration of bacterial surface protein architectures for guided assembly of cell populations. Presented at the American Chemical Society 253rd National Meeting; 2017 Apr; San Francisco, CA.

List of Symbols, Abbreviations, and Acronyms

AFM	atomic force microscopy
AMC	US Army Materiel Command
ARL	US Army Research Laboratory
Au	gold
AuNP	gold nanoparticles
DOD	Department of Defense
eCPX	coat protein
FimH	fimbrial protein
MFC	microbial fuel cell
RDECOM	US Army Research, Development and Engineering Command
Si	silicon
XPS	X-ray photoelectron spectroscopy

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